

University of Groningen

**Influence of beverage composition on the results of erosive potential measurement by different measurement techniques**

Jager, D. H. J.; Vieira, A. M.; Ruben, J. L.; Huysmans, M. C. D. N. J. M.

*Published in:*  
CARIES RESEARCH

*DOI:*  
[10.1159/000116118](https://doi.org/10.1159/000116118)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2008

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Jager, D. H. J., Vieira, A. M., Ruben, J. L., & Huysmans, M. C. D. N. J. M. (2008). Influence of beverage composition on the results of erosive potential measurement by different measurement techniques. *CARIES RESEARCH*, 42(2), 98-104. <https://doi.org/10.1159/000116118>

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

# Influence of Beverage Composition on the Results of Erosive Potential Measurement by Different Measurement Techniques

D.H.J. Jager A.M. Vieira J.L. Ruben M.C.D.N.J.M. Huysmans

Academic Center for Oral Health, University Medical Center Groningen, Groningen, The Netherlands

## Key Words

Acidic beverages · Calcium analysis · Erosion · Inorganic phosphorus analysis · Mineral composition · Optical profilometry

## Abstract

The influence of beverage composition on the measurement of erosive potential is unclear. The aim of this study was to evaluate whether beverage composition influences the measurement of erosive potential and to evaluate the influence of exposure in small and large volumes. Eleven beverages were included: water (control), 3 alcopops, 2 beers and 5 soft drinks. For each beverage 15 bovine enamel samples were used: 5 for chemical and 10 for profilometric analysis. After exposure to the beverages (63 min) the resulting solutions were analyzed for Ca and inorganic phosphorus ( $P_i$ ) content. The samples for optical profilometry were submerged sequentially in 500 ml or in 1 ml of the drinks for 3, 6, 9, 15 and 30 min (total 63 min). For some of the beverages high baseline concentrations of Ca (energy drink) or  $P_i$  (cola drink, cola lemon drink, beer, beer lemon) were found. Some of the beverages showed a good correlation between the chemical methods. Profilometry (both for 1 and 500 ml) showed generally lower enamel losses than the chemical methods. Lower enamel losses were found for the profilometry 1 ml compared to the profilometry 500 ml only for the cola drinks. It can be concluded that the composition of the beverages had a significant effect on the determination of

the erosive potential with chemical analyses. Drink composition also influenced the effect of small versus large exposure volumes, indicating the need for standardization of exposure parameters.

Copyright © 2008 S. Karger AG, Basel

Dental erosion is defined as an irreversible loss of dental hard tissue due to a chemical process without involvement of microorganisms [Imfeld, 1996]. Dental erosion may be caused by either extrinsic or intrinsic factors. One of the extrinsic causes of dental erosion is excessive consumption of acidic beverages [Dugmore and Rock, 2004]. Different techniques are available to assess the erosive potential of acidic beverages. Frequently used techniques include profilometry and chemical analysis.

Calcium determination and inorganic phosphorus ( $P_i$ ) determination are used to measure the loss of minerals from the enamel and are well established and accurate [Barbour and Rees, 2004]. Small concentrations of ions released from the enamel can be measured, so it is possible to observe the initial stages of erosion and to use small volumes of the examined solutions. It also allows the use of natural tooth surfaces since polishing is not needed [Barbour and Rees, 2004]. However, chemical methods provide only quantitative and not morphological or mechanical data [Grenby, 1996].

For measuring the loss of surface layers the method of choice is optical or contact profilometry. In optical pro-

**Table 1.** Beverages used in this study, with details of their composition

	Beverage	Producer	pH	Ca, mmol/l	P <sub>i</sub> , mmol/l	DS (HA)
Cola drink	Coca Cola	Coca-Cola Enterprises Nederland BV, Dongen, The Netherlands	2.47	0.87 ± 0.04	4.76 ± 0.15	0.005
Cola lemon drink	Coca Cola light lemon	Coca-Cola Enterprises Nederland BV, Dongen, The Netherlands	2.73	0.73 ± 0.01	4.90 ± 0.06	0.008
Orange drink	Fanta orange	Coca-Cola Enterprises Nederland BV, Dongen, The Netherlands	3.03	0.06 ± 0.01	0.19 ± 0.01	0.001
Fruit drink	Dubbel friss orange/ pink grapefruit	Riedel Beverages, Ede, The Netherlands	3.35	1.30 ± 0.03	0.51 ± 0.03	0.018
Vodka alcopop	Smirnoff Ice	Diageo, London, UK	3.43	0.15 ± 0.005	0.004 ± 0.001	<0.001
Energy drink	Red Bull	Red Bull, de Bilt, The Netherlands	3.43	2.40 ± 0.21	0.01 ± 0.02	<0.001
Ice tea	Lipton Ice tea	Unilever, Rotterdam, The Netherlands	3.80	0.12 ± 0.01	0.25 ± 0.004	0.009
Beer lemon	Grolsch beer lemon	Inbev Nederland, Breda, The Netherlands	3.83	0.96 ± 0.02	3.51 ± 0.08	0.068
Rum lime alcopop	Breezer Lime	Bacardi Martini NV, Gouda, The Netherl.	3.87	0.17 ± 0.01	0.02 ± 0.001	<0.001
Beer	Bavaria beer	Bavaria NV, Lieshout, The Netherlands	4.20	0.72 ± 0.02	5.30 ± 0.14	0.125
Bottled water	Sourcy bottled water	Vrumona BV, Bunnik, The Netherlands	8.09	1.20 ± 0.03	n.m.	–

DS = Degree of saturation; HA = hydroxyapatite; n.m. = not measured.

filometry there is no physical contact between the probe and the surface, so no damage will occur by scratching of the softened surface [Barbour and Rees, 2004]. A drawback of profilometric techniques is that enamel losses below 2 µm are difficult to measure.

Little is known about the correlation between different methods and the lack of a 'gold standard' is a shortcoming in the field of erosion research. Moreover, the influence of the composition of the beverages on the measurements is unclear. In earlier studies comparing methods for measuring the erosive potential of beverages, standard solutions such as citric acid or lactate buffer were used [Ganss et al., 2005; Zero et al., 1990]. As a result, the influence of the composition of beverages on the results obtained by the different methods could not be determined. We hypothesized that the chemical composition of soft drinks and the volume used influence the determination of erosive potential.

The aim of this study was to evaluate whether beverage composition influences the measurement of erosive potential and to evaluate the influence of exposure in small and large volumes.

## Materials and Methods

### Preparation of Samples

A total of 165 buccal surfaces of extracted bovine incisors, stored in water, were ground flat with water-cooled silicon carbide 220 grit grinding discs (SIA siawat P220, Frauenfeld, Swit-

zerland) and cut into blocks of approximately 5 × 3 mm using a vertical sawing machine with a diamond saw blade (11-4243, Buehler, Düsseldorf, Germany). The blocks were embedded in acrylic resin (Autoplast polymer, Candulor AG, Wangen, Switzerland) leaving the enamel surface uncovered and subsequently the samples were polished flat (800–1,200 grit grinding paper) and thoroughly rinsed with tap water. The samples were randomly divided into three groups of 55 samples each: one group for chemical analysis and two groups for profilometric analysis.

Before inclusion in the experiment the area of exposure of each of the 55 samples used for chemical analyses was measured with a stereomicroscope equipped with a measuring grid (Leitz Durimet, Wetzlar, Germany) fitted out with a digital xy table (Sony Magnescale LY101, Tokyo, Japan).

The 110 samples used for the profilometric analysis were partly covered with PVC tape exposing an area of approximately 3 × 3 mm in the center of the enamel sample.

### Beverages

Eleven beverages, all available in the Netherlands, were included in this study (table 1). Immediately after opening the bottles, and also after degassing (the drinks were placed on a shaking table set at 200 rpm until no bubbles were visible), the pH was measured 5 times using a calibrated glass pH electrode (Radiometer, PHM 84 Research Meter, G202C, Copenhagen, Denmark) in 100 ml of the beverages. Room temperature in the laboratory was 21°C with a possible variation of ±2°C. Standard buffers, pH 7.01 and 4.00 (20°C), were used (measurement uncertainty for both ±0.015 units) (Merck KGaA, Darmstadt, Germany). Calibration was performed with these buffers every day.

### Demineralization Procedures

Before starting the demineralization procedure the samples for chemical analysis were submersed for 3 min in 3 ml of a standard solution of 50 mM citric acid, 0.4 mM KH<sub>2</sub>PO<sub>4</sub>, 0.4 mM CaCl<sub>2</sub>

and 1 mM  $\text{NaN}_3$  (pH = 3) to remove the smear layer from the polished surfaces and subsequently rinsed with tap water. All the beverages were decarbonated.

For the chemical analyses each of the 5 enamel samples was submersed in 1 ml of each beverage in a test tube for 3 min under constant agitation on a shaking table at 100 rpm. After 3 min the samples were lifted from the beverages and the enamel surface was rinsed with 3 ml of demineralized water which was collected in the test tube. From the resulting mixtures 1 ml was used for Ca analysis and 1 ml for  $\text{P}_i$  analysis. The same procedure was repeated for exposures of 6, 9, 15 and 30 min (total 63 min). The exposures for the different times were made sequentially on the same specimens.

For the profilometric analysis each of the 5 enamel samples was submersed in 1 ml of each beverage in a test tube for 3, 6, 9, 15 and 30 min under constant agitation on a shaking table (100 rpm). The pH of these solutions was measured after each exposure. Another set of 5 samples was submersed in 500 ml of each beverage for 63 min under constant agitation on a shaking table (100 rpm) in beakers with a diameter of 9.5 cm. All experiments were performed at room temperature ( $21 \pm 2^\circ\text{C}$ ).

#### *Chemical Analysis*

$\text{P}_i$  concentration in the beverages was determined using a phosphomolybdate spectrophotometric method [Chen et al., 1956]. The concentration of Ca in the beverages was determined by atomic absorption spectroscopy (PerkinElmer Analytical Instruments, Shelton, Conn., USA) [Vieira et al., 2005]. This was performed in the presence of lanthanum (0.326%) in order to suppress phosphate interference. An air/ $\text{C}_2\text{H}_2$  flame and a wavelength of 422.7 nm were used.

For the chemical analyses all the beverages had to be diluted with demineralized water. For  $\text{P}_i$  analysis most of the beverages were diluted 16 times in total. The beverages with high  $\text{P}_i$  concentrations (the colas and the beers) had to be diluted 80 times. For Ca analysis all the beverages were diluted 18.4 times.

The Ca and  $\text{P}_i$  losses from the enamel samples were determined by subtracting the Ca or  $\text{P}_i$  content of the beverages before the enamel exposure (average of 10 measurements) from the total Ca or  $\text{P}_i$  content of the solution after exposure. In addition, the ratio of the Ca dissolved to the  $\text{P}_i$  dissolved ( $\Delta\text{Ca}/\Delta\text{P}$ ) was calculated for each exposure time.

Lesion depth was calculated from the Ca and  $\text{P}_i$  loss using the average Ca and  $\text{P}_i$  content per unit volume for bovine enamel and the exposed enamel area [Dijkman et al., 1983]. A Ca concentration in enamel of 25.1%, a P concentration in enamel of 17.61% were assumed and an average enamel density of  $2.93 \text{ g/cm}^3$  was assumed. This resulted in two depth parameters: d(Ca) and d(P), lesion depth estimated from Ca loss or  $\text{P}_i$  loss, respectively. The estimated erosion depth ( $\mu\text{m}$ ) of the 5 samples was averaged.

#### *Profilometric Analysis*

Erosion depths were measured using an optical profilometer (Proscan 2000, Scantron, Taunton, England). Before inclusion of the enamel samples in the experiment, baseline measurements were performed on each sample in order to confirm the flatness of the polished enamel surfaces.

After the demineralization procedure the PVC tape was removed. The samples were scanned over the reference and eroded surfaces. The volume lost due to erosion was calculated with the

equipment's software. Erosion depth ( $\mu\text{m}$ ) was calculated by dividing the volume loss by the exposed enamel area of the scanned surface. The erosion depths of the 5 samples were averaged. The profilometry resulted in two further depth parameters: d(prof1) and d(prof500).

#### *pH Changes and Degree of Saturation*

The pH of the solutions after each exposure in the profilometry (1-ml) group was measured. The baseline degree of saturation of the beverage with regard to hydroxyapatite and dicalcium phosphate dihydrate (DCPD) was calculated by means of a computer program [Shellis, 1988], using the baseline Ca and  $\text{P}_i$  concentrations of the beverages, together with the pH measured after degassing. To determine the possible influence of saturation of the beverages on the measurement results during the erosion process, the Ca and  $\text{P}_i$  concentrations and pH after the 30-min incubation were used to calculate the change in degree of saturation with regard to hydroxyapatite and DCPD after the erosion regime.

#### *Statistical Analysis*

For investigation of the relationship between the change in Ca and  $\text{P}_i$  concentrations, linear least squares regression was performed. The  $\text{P}_i$  concentration was the independent (x) variable. A one-way ANOVA followed by a Bonferroni post-hoc test in SPSS 12.01 (SPSS, Chicago, Ill., USA) was used to test differences between the cumulative erosive depths at 63 min obtained by the chemical methods [average of d(Ca) and d(P): d(CaP), d(prof1) and the d(prof500)]. The significance level for all tests was set at 0.05.

## **Results**

The pH of the beverages ranged from 2.4 (cola) to 8.1 (bottled water) (table 1). Table 1 also shows the baseline Ca and  $\text{P}_i$  concentrations and table 2 shows the changes in Ca and  $\text{P}_i$  concentrations for all erosion times and all drinks.  $\text{P}_i$  concentration ranged from not detectable (bottled water) to 5.3 mmol/l (beer). Baseline Ca concentration ranged from 0.06 mmol/l (orange soft drink) to 1.3 mmol/l (fruit drink). For most of the drinks the  $\Delta\text{Ca}/\Delta\text{P}$  ratio did not differ significantly from 1.6 except for some of the low exposure times (3 and 6 min), and for the cola drink, orange soft drink, and the ice tea. In table 3, the parameters for the linear least squares regression analysis of the Ca and the  $\text{P}_i$  concentrations for all beverages are presented. In most cases a high linear correlation ( $r^2 > 0.8$ ) was found, except for the beers ( $r^2 = 0.07$  and  $r^2 = 0.19$ ), cola drink ( $r^2 = 0.76$ ), energy drink ( $r^2 = 0.63$ ) and cola lemon drink ( $r^2 = 0.53$ ). For this reason and because of the problems measuring the  $\text{P}_i$  concentration in drinks with a high baseline  $\text{P}_i$  concentration, d(Ca) of the beers, cola drink and cola lemon was used for the comparison with profilometry. In table 4 and in figure 1, the cumula-

**Table 2.** Changes in calcium (Ca) and inorganic phosphorus (P<sub>i</sub>) concentrations (mmol/l) of drinks after each exposure, together with the ratio of the changes in Ca and P<sub>i</sub> ( $\Delta\text{Ca}/\Delta\text{P}_i$ )

	3 min			6 min			9 min			15 min			30 min			DS (HA)
	Ca	P <sub>i</sub>	Ca/P <sub>i</sub>	Ca	P <sub>i</sub>	Ca/P <sub>i</sub>	Ca	P <sub>i</sub>	Ca/P <sub>i</sub>	Ca	P <sub>i</sub>	Ca/P <sub>i</sub>	Ca	P <sub>i</sub>	Ca/P <sub>i</sub>	
Cola drink	0.15 (0.02)	0.15 (0.05)	1.16 (0.36)	0.26 (0.02)	0.26 (0.09)	1.22 (0.84)	0.32 (0.05)	0.33 (0.26)	1.89 (1.49)	0.42 (0.05)	0.61 (0.12)	0.70 (0.13)	0.61 (0.05)	0.87 (0.06)	0.71 (0.05)	0.009
Cola lemon drink	0.17 (0.03)	0.26 (0.09)	0.71 (0.32)	0.25 (0.02)	0.25 (0.15)	1.81 (1.85)	0.33 (0.03)	0.20 (0.10)	1.97 (0.91)	0.47 (0.02)	0.17 (0.07)	3.16 (1.17)	0.85 (0.02)	0.74 (0.18)	1.21 (0.31)	0.001
Orange soft drink	0.13 (0.02)	0.05 (0.01)	2.47 (0.56)	0.14 (0.03)	0.10 (0.02)	1.37 (0.15)	0.19 (0.05)	0.15 (0.04)	1.30 (0.12)	0.26 (0.05)	0.26 (0.06)	1.03 (0.03)	0.48 (0.10)	0.53 (0.12)	0.90 (0.04)	0.007
Fruit drink	0.17 (0.04)	0.07 (0.03)	2.67 (0.59)	0.29 (0.05)	0.15 (0.03)	2.06 (0.66)	0.37 (0.04)	0.17 (0.03)	2.17 (0.28)	0.46 (0.07)	0.26 (0.04)	1.76 (0.12)	0.94 (0.15)	0.50 (0.09)	1.90 (0.32)	0.029
Vodka alcopop	0.13 (0.02)	0.06 (0.02)	2.14 (0.33)	0.21 (0.02)	0.11 (0.01)	1.97 (0.17)	0.28 (0.04)	0.15 (0.02)	1.84 (0.07)	0.36 (0.04)	0.22 (0.03)	1.66 (0.06)	0.71 (0.14)	0.46 (0.09)	1.54 (0.03)	0.017
Energy drink	-0.06 (0.05)	0.05 (0.01)	-1.18 (0.97)	0.00 (0.10)	0.02 (0.10)	-0.07 (1.25)	0.17 (0.20)	0.15 (0.04)	1.06 (1.38)	0.47 (0.15)	0.22 (0.05)	2.15 (0.69)	0.83 (0.11)	0.46 (0.09)	1.83 (0.19)	0.032
Ice tea	0.11 (0.01)	0.04 (0.001)	2.48 (0.23)	0.15 (0.01)	0.06 (0.01)	2.34 (0.29)	0.20 (0.02)	0.09 (0.01)	2.19 (0.09)	0.21 (0.03)	0.12 (0.02)	1.74 (0.09)	0.37 (0.12)	0.28 (0.02)	1.32 (0.40)	0.024
Beer lemon	0.09 (0.04)	-0.02 (0.10)	1.75 (4.12)	0.07 (0.04)	-0.14 (0.22)	2.48 (4.23)	0.11 (0.04)	-0.02 (0.10)	-0.82 (3.23)	0.12 (0.04)	-0.17 (0.17)	0.28 (2.54)	0.21 (0.04)	0.03 (0.19)	2.84 (4.08)	0.077
Rum lime alcopop	0.13 (0.02)	0.05 (0.01)	2.78 (0.32)	0.17 (0.02)	0.07 (0.02)	2.53 (0.41)	0.21 (0.06)	0.09 (0.03)	2.33 (0.33)	0.28 (0.07)	0.15 (0.03)	1.81 (0.14)	0.48 (0.10)	0.28 (0.07)	1.72 (0.10)	0.028
Beer	0.10 (0.02)	-0.09 (0.07)	-4.12 (7.07)	0.09 (0.01)	-0.08 (0.13)	-1.26 (2.42)	0.10 (0.02)	-0.02 (0.10)	1.80 (3.29)	0.05 (0.06)	-0.13 (0.07)	-0.17 (0.88)	0.11 (0.03)	0.27 (0.13)	0.51 (0.42)	0.146
Bottled water	-0.02 (0.01)	n.m.	n.m.	-0.01 (0.02)	n.m.	n.m.	-0.03 (0.02)	n.m.	n.m.	-0.01 (0.04)	n.m.	n.m.	-0.02 (0.03)	n.m.	n.m.	-

Means with SD in parentheses. The degree of saturation (DS) with respect to hydroxyapatite (HA) after the final 30-min exposure is also given. n.m. = Not measured.

tive results of Ca, P<sub>i</sub> and the profilometric analyses are presented. The highest enamel loss was found for cola lemon drink in the d(prof500) group (13.54  $\mu\text{m}$ ). The drinks concentrating in the middle part of the graph (dashed lines) showed lower erosive potential for profilometry compared to the chemical analysis. The two colas showed lower erosive potential in the d(prof1) group compared to the d(prof500) group and higher erosive potential for the d(P<sub>i</sub>) compared to the d(Ca). In figure 1, also the rank order in which the different methods placed the drinks can be assessed. For the beverages the influence of the measurement method on its rank order in erosiveness (1 is lowest, 11 is highest erosion) is marked, e.g., the orange soft drink is the 7th most erosive drink in d(Ca) but the 4th most erosive with d(P<sub>i</sub>); similarly, ice tea is 8th with d(Ca) and 4th with d(prof500). One-way ANOVA showed a significant effect of measuring technique ( $p < 0.05$ ) for all beverages except ice tea and the fruit drink. d(CaP) showed an enamel loss that was significantly higher ( $p < 0.05$ ) than that of d(prof1) for the rum lime alcopop ( $p < 0.0001$ ), energy drink ( $p = 0.007$ ), vodka alcopop ( $p = 0.034$ ), beer [ $p < 0.0001$ , d(Ca) only] and orange soft drink ( $p < 0.0001$ ). When compared to

**Table 3.** Parameters for the linear least squares regressions of Ca concentration on P<sub>i</sub> concentration for all beverages

	Slope	y intercept	r <sup>2</sup>
Cola drink	0.47	0.14	0.75
Cola drink lemon	0.75	0.17	0.53
Orange soft drink	0.76	0.07	0.98
Fruit drink	1.69	0.06	0.91
Vodka alcopop	1.45	0.05	0.99
Energy drink	2.17	0.14	0.86
Ice tea	1.07	0.08	0.80
Beer lemon	0.10	0.44	0.19
Rum lime alcopop	1.49	0.06	0.97
Beer	0.05	0.09	0.07
Bottled water	6.94	0.09	0.18

The P<sub>i</sub> concentration was the independent (x) variable.

the d(prof500) the d(CaP) showed a significantly lower enamel loss for the cola lemon drink ( $p = 0.004$ ), and a significantly higher enamel loss was found for rum lime alcopop ( $p < 0.0001$ ), energy drink ( $p = 0.022$ ), vodka alcopop ( $p = 0.034$ ), beer ( $p < 0.0001$ ), beer lemon ( $p =$



**Table 4.** Cumulative loss of enamel after 63-min total exposure to the beverages

	d(Ca)	d(P <sub>i</sub> )	d(profl)	d(prof500)
Cola drink	4.44 ± 0.22	9.22 ± 1.25	2.08 ± 0.58	8.04 ± 3.62
Cola lemon drink	6.72 ± 0.36	8.97 ± 1.75	6.42 ± 1.15	13.54 ± 4.31
Orange soft drink	3.64 ± 0.22	5.50 ± 0.38	2.29 ± 0.88	2.37 ± 0.51
Fruit drink	6.55 ± 0.53	5.67 ± 0.72	4.24 ± 2.53	3.27 ± 1.17
Vodka alcopop	5.00 ± 0.85	4.94 ± 0.88	2.98 ± 1.06	2.69 ± 1.35
Energy drink	4.25 ± 0.96	4.84 ± 0.18	2.34 ± 0.85	2.69 ± 0.96
Ice tea	3.23 ± 0.48	3.11 ± 0.33	1.80 ± 1.34	3.08 ± 0.63
Beer lemon	1.99 ± 0.32	-1.79 ± 2.02	1.12 ± 0.98	0.00
Rum lime alcopop	4.09 ± 0.31	3.43 ± 0.29	0.84 ± 0.70	1.47 ± 0.72
Beer	1.30 ± 0.23	-0.38 ± 1.16	0.00	0.00
Bottled water	-0.33 ± 0.92	0.01 ± 0.01	0.00	0.00

Means with SD.

0.001) and orange soft drink ( $p = 0.001$ ). The d(profl) showed a significantly lower enamel loss than the d(prof500) only for the cola drink ( $p = 0.002$ ) and the cola lemon drink ( $p = 0.003$ ).

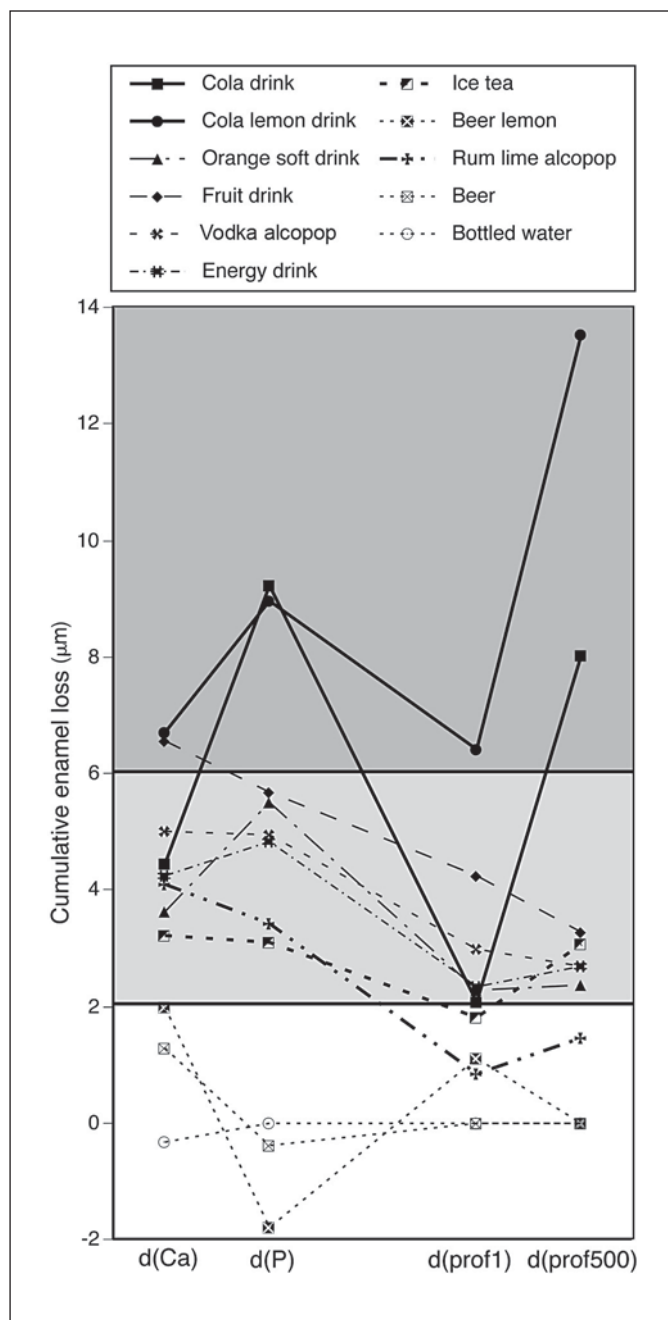
The results obtained for the pH measurements after each exposure in the 1-ml profilometry showed very little change in pH ( $-0.02$  to  $+0.03$ ) after the erosion process for most beverages. Only the cola and the cola lemon showed a small rise in pH (0.1) after 30 min. None of the beverages was supersaturated with respect to hydroxyapatite or DCPD after a 30-min erosive exposure in 1 ml of the samples (table 2). The highest degree of saturation for hydroxyapatite was found for the beers. The highest rise in the degree of saturation after 30 min was found for the energy drink.

## Discussion

In this study bovine enamel was used. Most in vitro studies use bovine enamel since it is considered a suitable substitute for human enamel [Zero, 1996]. Although Meurman and Frank [1991] did not observe any difference in the progression of erosion or the surface ultrastructure of erosive lesions between bovine and human prismatic enamel, another study showed that morphological differences such as a higher porosity exist when compared to human enamel, which results in higher rates of artificial caries lesion formation [Featherstone and Mellberg, 1981]. It should be considered that in this study a comparison between the methods was performed and not an extrapolation of the results to the clinical situation.

The P<sub>i</sub> analysis of the beers and the Ca analysis of the energy drink yielded negative values. For these beverages the differences between the Ca analysis and the P<sub>i</sub> analysis and the negative values may possibly be explained by their chemical composition. Because of the high baseline concentrations of Ca and P<sub>i</sub> it was sometimes necessary to use high dilutions which may have increased the measurement error. A high concentration of P<sub>i</sub> in the colas and the beers could also have interfered with the Ca measurements by calcium binding, but this was prevented by adding a high concentration of lanthanum. However, the amount of Ca and P<sub>i</sub> released from the enamel into solution by erosion was for some beverages relatively small compared to the baseline concentration of Ca or P<sub>i</sub> in the beverages, especially for the short exposure times. This resulted in small changes in mineral concentration, which did not always exceed the measurement error and made it difficult to obtain reliable measurements.

Previous studies, using standard erosive solutions such as citric acid or lactate buffer reported almost perfect agreement between the Ca and P<sub>i</sub> analyses [Ganss et al., 2005; Zero et al., 1990]. Because of the use of standard solutions in these studies the influence of the composition of the erosive solution on the results could not be determined. In the present study, for three of the drinks a Ca/P ratio (table 2) with significant deviations from 1.6 (the Ca/P ratio of bovine enamel) was found. This suggests that reprecipitation occurred during the erosive exposure. However, in none of the drinks could a supersaturation with respect to other calcium phosphates be calculated. No explanation could be found for the phenomenon which, especially for the orange drink, does



**Fig. 1.** Cumulative loss of enamel as measured by the four techniques, showing both the quantitative loss and the rank order for each beverage. To facilitate comparison between the techniques for each beverage, the points have been connected. The plot area is divided into three areas: little or no erosion (–2 to 2 µm), moderate erosion (2–6 µm), and severe erosion (6–14 µm).

seem to be systematic, looking at the decline of the Ca/P ratio with increasing exposure time. The short exposures and the drinks with low erosive potential were found to be irrelevant for the calculation of the Ca/P ratio because of the low enamel losses.

Profilometric analysis showed a trend for lower enamel loss compared to Ca and P<sub>i</sub> analysis. These results are in agreement with the findings of a previous study where Ca/P<sub>i</sub> analysis and contact profilometry were compared [Ganss et al., 2005]. The difference found between the chemical methods and the profilometry may be explained by the fact that the erosion process does not only remove enamel layers but also causes a ‘softening’. Profilometry does not account for the subsurface loss of the softened layer. The depth of the softened layer is unknown but may be more than 10 µm [Eisenburger et al., 2004].

Removal of reaction products and the supply of fresh acid are important for the continued formation of erosion lesions [Eisenburger and Addy, 2003]. In a study investigating the relationship between enamel erosion and liquid flow rate, it was concluded that the rate of erosion is dependent on liquid velocity, exposure time and the total volume of the acidic solution [Shellis et al., 2005]. In this study agitation of 1 ml probably results in a different replacement of liquid at the enamel surface compared to agitation of a 500-ml reservoir, thus influencing the erosion rate. However, regarding the exposure volume of the beverage, our study found significant differences between the measured erosive potential only for the two cola drinks.

Some authors have observed that a small change in the degree of saturation resulted in a difference in the dissolution of enamel and that it is an important parameter that defines the ability of a solution to demineralize enamel [Barbour et al., 2003; Finke et al., 2000; Margolis et al., 1999; Tanaka and Kadoma, 2000]. Only in a small volume of the beverages would a significant rise in saturation be expected. To ascertain whether this was the case in the present study, the saturation with respect to hydroxyapatite was determined for all 1-ml volumes before and after exposure. It was found that the degree of saturation rose with increasing exposure time. However, this was seen in all beverages and the highest rise was found for the energy drink. This did not explain our findings as only the cola drinks showed a lower erosion in the 1-ml exposure.

Although pH is a parameter in the calculation of the degree of saturation, it has been reported as a separate factor in erosive potential [Larsen and Nyvad, 1999; Margolis et al., 1999]. A rise in pH would result in a slowing

down of the erosion process. Only for the cola drink and the cola lemon drink a measurable, if low (0.1), rise in pH was found. As this corresponds to the observed reduced erosion for the two cola drinks in the 1-ml exposure model, we assume that pH was a determining factor.

In conclusion, the present study has shown that the composition of the beverages had a significant effect on the determination of the erosive potential with chemical analyses. This should be considered when choosing an

appropriate measurement method. Optical profilometry is suggested as a beverage-independent alternative. Beverage composition also influences the effect of small versus large exposure volumes, indicating the need for standardization of exposure parameters such as exposure times, volumes and flow rate of the drinks during exposure to prevent differences in erosion rate due to differences in liquid velocities.

## References

- Barbour ME, Parker DM, Jandt KD: Enamel dissolution as a function of solution degree of saturation with respect to hydroxyapatite: a nanoindentation study. *J Colloid Interface Sci* 2003;265:9–14.
- Barbour ME, Rees JS: The laboratory assessment of enamel erosion: a review. *J Dent* 2004;32: 591–602.
- Chen PS, Toribara TY, Warner H: Microdetermination of phosphorus. *Anal Chem* 1956; 28:1756–1758.
- Dijkman AG, Tak J, Jongebloed WL, Arends J: Influence of HClO<sub>4</sub> strength and etching time on rate of etching and surface roughness of human enamel. *Caries Res* 1983;17: 14–22.
- Dugmore CR, Rock WP: A multifactorial analysis of factors associated with dental erosion. *Br Dent J* 2004;196:283–286.
- Eisenburger M, Addy M: Influence of liquid temperature and flow rate on enamel erosion and surface softening. *J Oral Rehabil* 2003; 30:1076–1080.
- Eisenburger M, Shellis RP, Addy M: Scanning electron microscopy of softened enamel. *Caries Res* 2004;38:67–74.
- Featherstone JD, Mellberg JR: Relative rates of progress of artificial carious lesions in bovine, ovine and human enamel. *Caries Res* 1981;15:109–114.
- Finke M, Jandt KD, Parker DM: The early stages of native enamel dissolution studied with atomic force microscopy. *J Colloid Interface Sci* 2000;232:156–164.
- Ganss C, Lussi A, Klimek J: Comparison of calcium/phosphorus analysis, longitudinal microradiography and profilometry for the quantitative assessment of erosive demineralisation. *Caries Res* 2005;39:178–184.
- Grenby TH: Methods of assessing erosion and erosive potential. *Eur J Oral Sci* 1996;104: 207–214.
- Imfeld T: Dental erosion: definition, classification and links. *Eur J Oral Sci* 1996;104:151–155.
- Larsen MJ, Nyvad B: Enamel erosion by some soft drinks and orange juices relative to their pH, buffering effect and contents of calcium phosphate. *Caries Res* 1999;33:81–87.
- Margolis HC, Zhang YP, Lee CY, Kent RL Jr, Moreno EC: Kinetics of enamel demineralization in vitro. *J Dent Res* 1999;78:1326–1335.
- Meurman JH, Frank RM: Progression and surface ultrastructure of in vitro caused erosive lesions in human and bovine enamel. *Caries Res* 1991;25:81–87.
- Shellis RP: A microcomputer program to evaluate the saturation of complex solutions with respect to biominerals. *Comput Appl Biosci* 1988;4:373–379.
- Shellis RP, Finke M, Eisenburger M, Parker DM, Addy M: Relationship between enamel erosion and liquid flow rate. *Eur J Oral Sci* 2005; 113:232–238.
- Tanaka M, Kadoma Y: Comparative reduction of enamel demineralization by calcium and phosphate in vitro. *Caries Res* 2000;34:241–245.
- Vieira A, Ruben JL, Huysmans MC: Effect of titanium tetrafluoride, amine fluoride and fluoride varnish on enamel erosion in vitro. *Caries Res* 2005;39:371–379.
- Zero DT: Etiology of dental erosion – extrinsic factors. *Eur J Oral Sci* 1996;104:162–177.
- Zero DT, Rahbek I, Fu J, Proskin HM, Featherstone JD: Comparison of the iodide permeability test, the surface microhardness test, and mineral dissolution of bovine enamel following acid challenge. *Caries Res* 1990;24: 181–188.